UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

DTIC FILE COPY (1)

REPORT DOCUMENTATION PAGE

AD-A198 947

1b. RESTRICTIVE MARKINGS

3. DISTRIBUTION/AVAILABILITY OF REPORT
Approved for public release; distribution is unlimited

4. PERFORMING ORGANIZATION REPORT NUMBER(S)
NMRI 87-86

5. MONITORING ORGANIZATION REPORT NUMBER(S)

6a. NAME OF PERFORMING ORGANIZATION
Naval Medical Research

6b. OFFICE SYMBOL (If applicable)

7a. NAME OF MONITORING ORGANIZATION
Naval Medical Command

6c. ADDRESS (City, State, and ZIP Code)
Bethesda, Maryland 20814-5055

7b. ADDRESS (City, State, and ZIP Code)
Department of the Navy
Washington, D.C. 20372-5120

8a. NAME OF FUNDING/SPONSORING ORGANIZATION
Naval Medical Research and Development Command

8b. OFFICE SYMBOL (If applicable)

9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER

8c. ADDRESS (City, State, and ZIP Code)
Bethesda, Maryland 20814-5055

10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
61102A	3M161102BS10	AF 427	DA301616

11. TITLE (Include Security Classification)
PLASMODIUM FALCIPARUM: FREEZE-FRACTURE OF THE GAMETOCYTE PELLICULAR COMPLEX

12. PERSONAL AUTHOR(S)
C.A.M. Meszoely; E.F. Erbe; R.L. Steere; J. Trosper; R.L. Beaudoin

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Report No. 6	FROM 1986 TO 1987	1987	10

16. SUPPLEMENTARY NOTATION
Reprinted from: Experimental Parasitology 64: 300-309, 1987

17 COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)	
FIELD	GROUP	SUB-GROUP	Plasmodium falciparum	freeze-fracture
			parasitic protozoa	gametocyte
			human malaria	structure

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

DTIC ELECTE AUG 0 4 1988 S D

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	Unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
Phyllis Blum, Information Services Division	202-295-2188	ISD/ADMIN/NMRI

DD FORM 1473, 84 MAR — 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED

EXPERIMENTAL PARASITOLOGY 64, 300–309 (1987)

Plasmodium falciparum: Freeze-Fracture of the Gametocyte Pellicular Complex

CHARLES A. M. MESZOELY

Department of Biology, Northeastern University, Boston, Massachusetts 02115, U.S.A.

ERIC F. ERBE AND RUSSELL L. STEERE

Plant Virology Laboratory, U.S. Department of Agriculture, Beltsville, Maryland 20705, U.S.A.

AND

JAMES TROSPER AND RICHARD L. BEAUDOIN[1]

Infectious Diseases Program Center, Naval Medical Research Institute, Bethesda, Maryland 20814-5055, U.S.A.

(Accepted for publication 4 February 1987)

MESZOELY, C. A. M., ERBE, E. F., STEERE, R. L., TROSPER, J., AND BEAUDOIN, R. L. 1987. *Plasmodium falciparum:* Freeze-fracture of the gametocyte pellicular complex. *Experimental Parasitology* **64**, 300–309. Freeze-fracturing has been used to study the architecture of the pellicular complex of the gametocytes of *Plasmodium falciparum*. The gametocyte is surrounded by three membranes and a layer of subpellicular microtubules. During freeze-fracturing, each of the three membranes is split along its hydrophobic interior to yield a total of six fracture faces. The most obvious feature of each fracture face is the presence of globular intramembranous particles on their surfaces. The six fracture faces differ from one another in arrangement, size, and density of these intramembranous particles. In gametocytes, unlike in sporozoites, the intramembranous particles are always distributed randomly and lack any definite pattern or orientations. A unique feature of gametocytes revealed by the freeze-fracturing technique is the presence of several transverse sutures on the middle membrane that encircle the gametocyte and give it a segmented appearance.

INDEX DESCRIPTORS AND ABBREVIATIONS: *Plasmodium falciparum;* Protozoa, parasitic; Malaria, human; Freeze-fracture; Gametocyte; Structure; Protoplasmic face of vacuolar membrane (PV); External face of the vacuolar membrane (EV); External face of middle membrane (EM); Protoplasmic face of middle membrane (PM); Protoplasmic face of inner membrane (PI); External face of inner membrane (EI).

INTRODUCTION

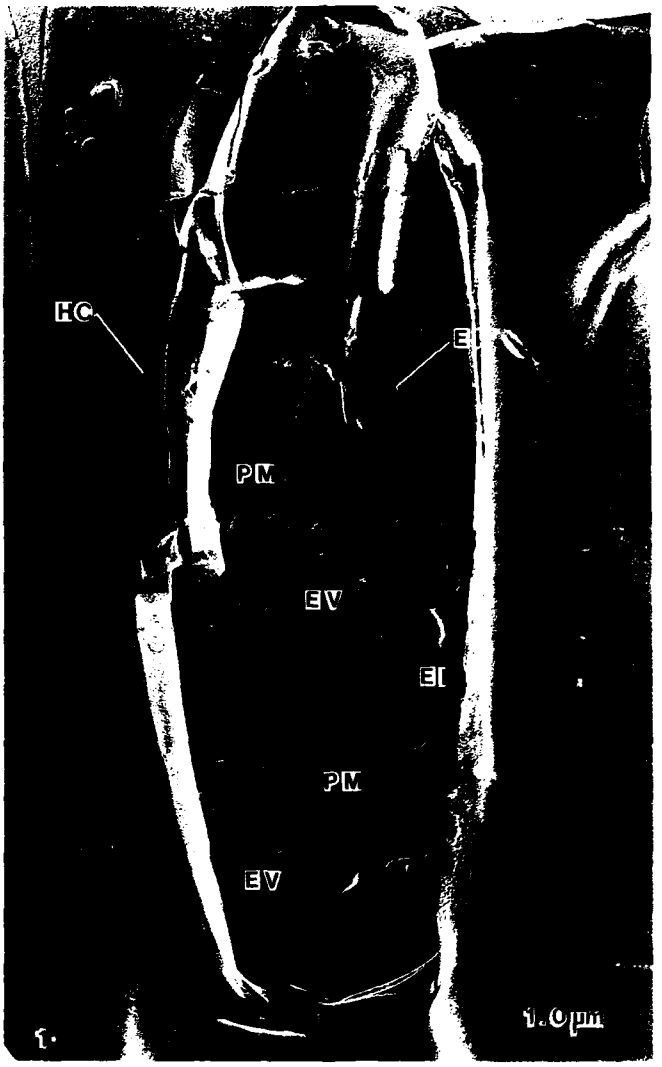
The first ultrastructural study on the gametocytes of the malarial parasite was carried out by Duncan and his colleagues in 1959 using the avian parasite *Plasmodium cathemerium*. Since that early report, several other transmission electron microscope studies have appeared. Garnham and his co-workers (1967) examined the fine structure of *P. berghei*, Rudzinska and Trager (1968) that of *P. coatneyi*, and Aikawa and Jordan (1968) that of *P. floridense*. More recently, Sterling and Aikawa (1973) reviewed the ultrastructure of the avian Haemosporidia, and Sinden and his co-workers (1976) studied the gametogenesis in the rodent malaria *P. yoelii*. All of these studies were limited to the exami-

[1] To whom correspondence should be addressed.

0014-4894/87 $3.00

nation of ultrathin stained sections except for that by Sinden (1975) where the scanning microscope was also used to examine microgametogenesis in the above species.

In contrast to the numerous conventional transmission electron microscopic studies on gametocytes of the malarial parasite, no detailed study to date has been carried out using the techniques of freeze-fracturing on the sexual stages of the life cycle. Freeze-fracturing furnishes detailed information about the membrane anatomy not obtain-



FIGS. 1–8. Abbreviations for all figures: PV, protoplasmic face of the *Plasmodium falciparum* vacuolar membrane; EV, external face of the vacuolar membrane; EO, external face of the outer membrane; PO, protoplasmic face of the outer membrane; PM, protoplasmic face of the middle membrane; EM, external face of the middle membrane; EI, external face of the inner membrane; PI, protoplasmic face of the inner membrane; HC, host cytoplasma; PF, protoplasmic face of the host erythrocyte membrane; FV, food vacuole; N, nucleus.

FIG. 1. Intraerythrocytic gametocyte of *Plasmodium falciparum* showing three fracture faces and transverse sutures (arrow) dividing the middle membrane of the parasite into at least nine segments.

able by other methods and has been used to elucidate the membrane architecture in several stages of the malaria parasite's life cycle: the asexual erythrocytic stage was studied by Ladda and Steere (1969), Seed *et al.* (1971), Meszoely *et al.* (1972), and McLaren *et al.* (1977, 1979), and exoerythrocytic stages by Meszoely *et al.* (1975). The mosquito stages have been examined by Dubremetz *et al.* (1979), Aikawa *et al.* (1979), and Meszoely *et al.* (1982). To date, the only freeze-fracture study on the gametocytes is a brief report by Meszoely *et al.* (1983). The present study was undertaken to describe in detail for the first time the architecture of the pellicular complex in gametocytes of the malaria parasite of man, *P. falciparum*.

Materials and Methods

The erythrocytic stages of the NF54 strain of *Plasmodium falciparum* obtained from the University of Nijmegen and the Brazilian clone 7G8 line provided by the Walter Reed U.S. Army Institute of Research were used as sources of *P. falciparum* parasites. Gametocytes were produced *in vitro* by static culture techniques slightly modified from those previously described (Ifediba and Vanderberg 1981). Parasites in asexual stages were killed by treatment with mitomycin C 3 days prior to fixation (Sinden *et al.* 1984). This provided pure cultures of nearly mature stage IV and mature stage V gametocytes.

The parasitized red blood cells were fixed in 2% glutaraldehyde and cryoprotected in 40% glycerol. This preparation was frozen and fractured in a modified Denton DFE-2 freeze-etch module. Both fracture faces of the specimen were shadowed simultaneously to obtain complementary replicas as described by Steere (1973). Stereo pairs with specimen tilt of 10° between electron micrographs were obtained with the JEM-100-B transmission electron microscope equipped with 60° top entry goniometer stage.

Results

The mature intraerythrocytic gametocytes, stages IV and V of *Plasmodium falciparum*, appear cigar shaped and sausage shaped, respectively, in freeze-fractured preparations filling most of the cytoplasm of the host erythrocyte (Figs. 1, 3, 6a, 7a, b). When the parasite is fractured transversely, it is roughly circular in outline. At this time, micro- and macrogametocytes cannot be readily differentiated from one another in freeze-fractured preparations.

The pellicular complex of the gametocyte consists of three membranes as well as a single row of subpellicular microtubules (Figs. 4, 8). During freeze-fracturing, each of the membranes fractures along its hydrophobic interior, yielding a total of six fracture faces. Intramembranous particles of different size and distribution cover

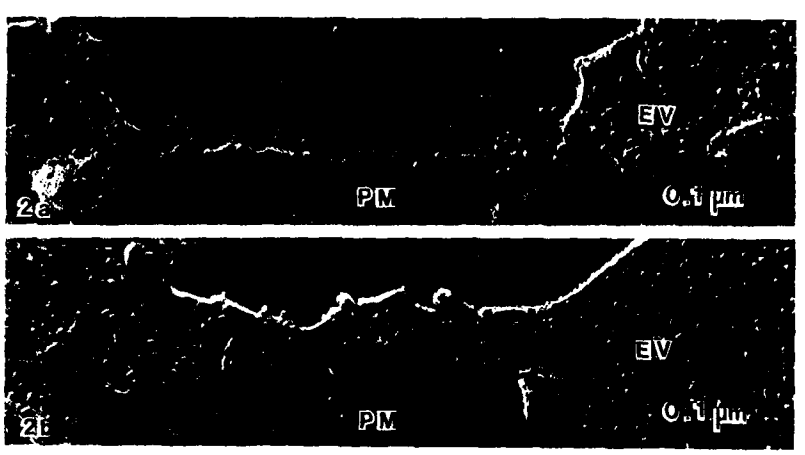


FIG. 2. *Plasmodium falciparum:* Gametocyte with transverse suture. (a) Transverse suture segmenting the middle membrane. (b) EV face of the outer membrane adhering to the suture and covering it. Note that the outer membrane shows no trace of the suture.

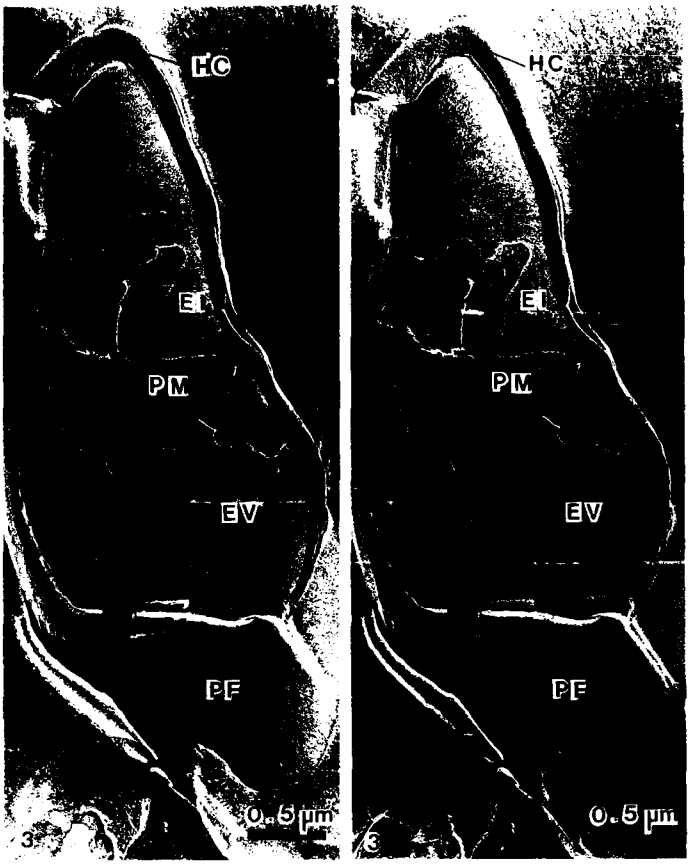


FIG. 3. *Plasmodium falciparum:* Gametocyte showing three fracture faces and also showing the protoplasmic face (PF) of the host erythrocyte. Suture (arrow).

these fracture faces (Figs. 4, 5). Nearly all the fracture faces differ from one another except EV and EI, which closely resemble one another. The nomenclature for labeling is based on that of Branton *et al.* (1975) and McLaren *et al.* (1979). The faces are described in order from the outside toward the interior of the gametocyte. The outermost membrane of the pellicular complex of the gametocyte is derived from the host erythrocyte, and consequently its fracture faces (PV and EV) are referred to as vacuolar. The outer plasma membrane of the host erythrocyte has been studied by freeze-fracture techniques by McLaren *et al.* (1977) and is not considered here.

The PV is covered by particles of approximately 10 nm in diameter. The density of these particles per unit area is about one-fourth the number seen on the opposing EV, which is densely covered with particles of approximately the same size as those found on PV. Particle size varies with most particles in the 10-nm range to a few down to 5 nm. The EM is covered by numerous particles of much smaller size (5 nm) than seen on the previous fracture faces PV and EV, but the density of these particles per unit area is about the same as observed for EV. The PM is nearly smooth with only a few widely scattered particles ranging in size from 5 to 10 nm present on

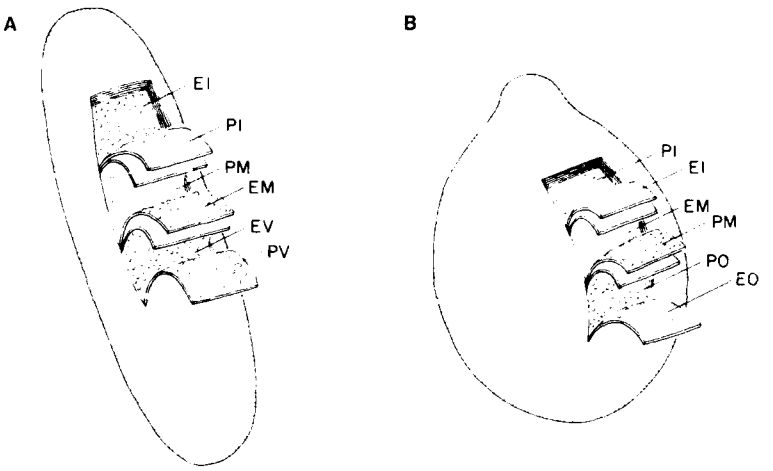


FIG. 4. *Plasmodium falciparum:* Diagrammatic reconstruction of the fracture faces of (A) the intraerythrocytic gametocyte (shown without the host erythrocyte) and (B) the free merozoite. Note that while the fracture faces of the outer membranes of the two stages look very similar, the two are not homologous. The outer membrane of the gametocyte is derived from the host erythrocyte while that of the merozoite is of parasite origin.

its surface. The PI is also nearly smooth, but the size of the few particles present is only in the 5 nm range. The EI resembles very closely that of EV in both the size and density of its particles.

A unique feature of the gametocyte visible only in freeze-fracture preparations is the presence of several transverse suture lines in the middle membrane that appear to divide the parasite into several segments (Figs. 1, 2, 3). Up to 12 segments and 11 sutures have been observed in a single parasite. The distance between sutures is rather uniform, measuring on the average around 0.7 μm. The suture is not present on either the outer or the inner membranes of the gametocyte, but its presence is marked on the inner membrane by a faint depression or groove. Also, the outer membrane shows strong affinity to the middle membrane along the suture line and remains attached to this area, even though the greater part of this membrane is fractured away (Fig. 2b).

In addition to the fracture faces of the membrane, subpellicular microtubules are also visible in crossfractured gametocyte preparations (Fig. 8), as well as other cell organelles including nuclei (Fig. 7), cytostome (Fig. 5a), and food vacuoles (Fig. 8).

DISCUSSION

A pellicular complex consisting of three membranes and row of subpellicular microtubules can be clearly identified in the freeze-fractured preparations of *Plasmodium falciparum* gametocytes. The six fracture faces resulting from the splitting of these membranes are distinguishable from one another by size and number of the intramembranous particles on their surfaces.

Unlike sporozoites of *P. yoelii*, *P. berghei*, and *P. knowlesi* (Dubremetz *et al.* 1979; Aikawa *et al.* 1979; Meszoely *et al.* 1982) and trophozoites of *P. knowlesi* (McLaren *et al.* 1979), the intramembranous particles of the gametocytes of *P. falciparum* are not organized into a distinct pattern but are randomly distributed on all six of its fracture faces. The pellicular system of the gametocyte in regard to particle distribution surprisingly resembles that of the free merozoite (McLaren *et al.* 1979). This is unexpected since the free

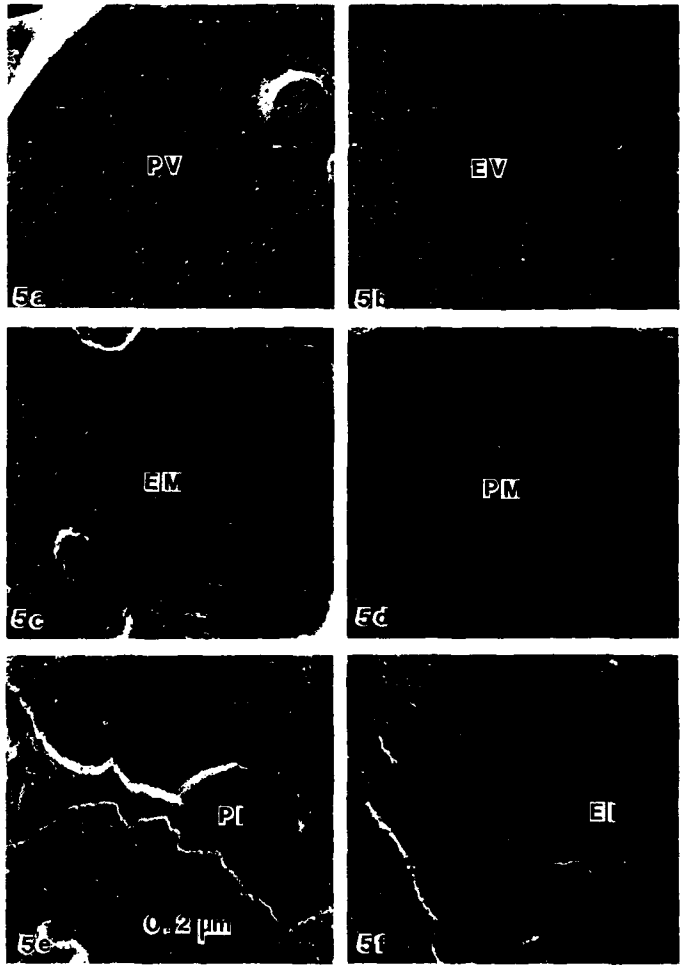


FIG. 5. *Plasmodium falciparum:* The six fracture faces of the gametocyte arranged from (a) to (f) in order toward the interior of the gametocyte. The plug-like structure in the right hand corner of (a) is a cytostome.

merozoite's outer membrane is presumed to become the gametocyte's middle membrane as the parasite invades the erythrocyte and carries the red cell's plasma membrane with it, transforming it into the outer or vacuolar membrane of the pellicular complex of the gametocyte (Sterling and Aikawa 1973). Little is known about the early development of the gametocyte, but considerable rearrangement of intramembranous particles would have to take place in these membranes for them to be homologous. Also, the outer or vacuolar membrane of the gametocyte bears little resemblance to that of the trophozoite, and the two are regarded as homologous. These observations strongly suggest that major rearrangements take place in the pellicular complex when the malarial parasite enters another stage of its life cycle, and that the architecture of the pellicular system is stage specific and is most likely related to a specific function and location in the host.

Another striking morphological feature

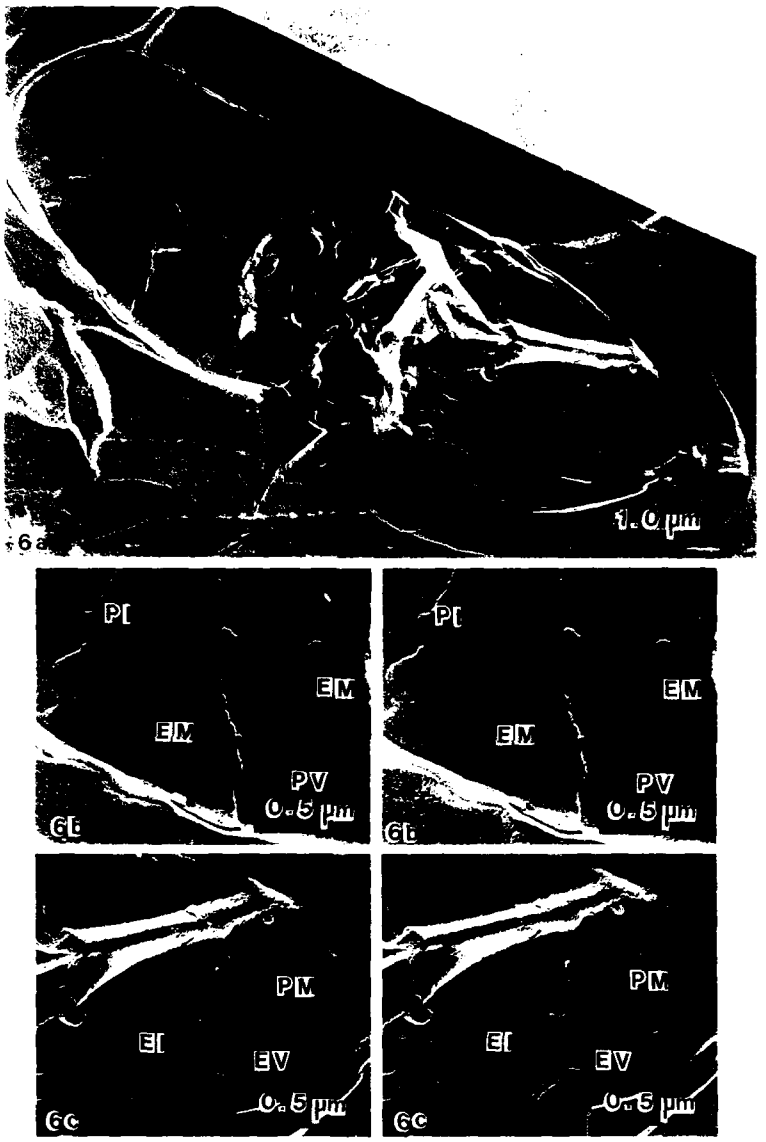


FIG. 6. *Plasmodium falciparum:* (a) Gametocyte showing all six fracture faces and transverse sutures. (b) Stereo pair of the above parasite showing the area to the left. (c) Stereo pair of the area to the right.

found was a series of transverse sutures on the gametocyte middle membrane. These sutures subdivide the middle membrane into several ring-like plates giving the parasite a segmented appearance. The transverse sutures on the gametocyte are unlike the single longitudinal suture reported for the sporozoite (Meszoely *et al.* 1982) both in their appearance and in their orientation. In the sporozoite, only a single suture occurs, which runs parallel to the long axis of the parasite, whereas in the gametocyte, up to 11 of these transverse sutures have been observed, and they are always ori-

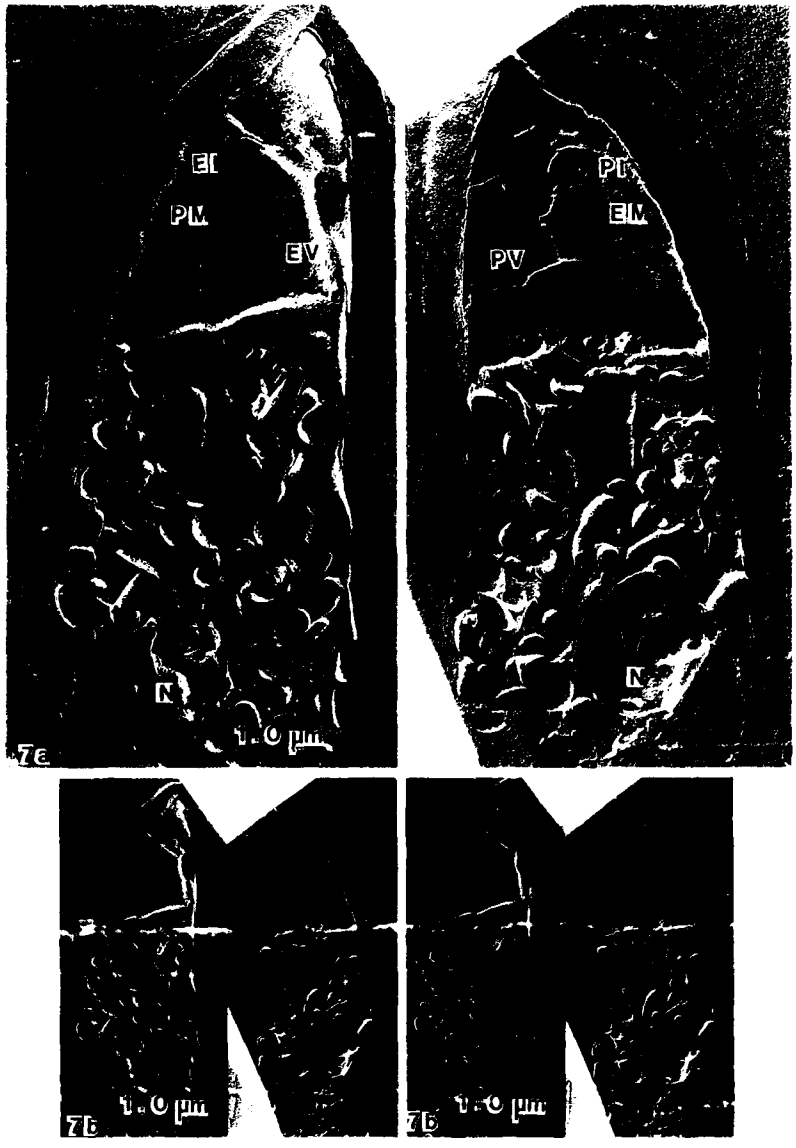


FIG. 7. *Plasmodium falciparum*: (a) Complementary replica of a gametocyte showing all six fracture faces and some of its organelles. (b) Stereo complementary pair of the same.

ented at a right angle to the long axis of the parasite. Also, the longitudinal suture in the sporozoite involves both the middle and the inner membrane, whereas the transverse ring sutures of the gametocyte are solely on the middle membrane. It is interesting to note that pieces of the outer membrane frequently remain attached to the middle membrane in the vicinity of the suture, while most of the remaining outer membrane fractures away, which suggests some special affinity the outer membrane has for the middle membrane at the level of the suture.

As pointed out above, evidence from this and other freeze-fracture studies indicates

FIG. 8. *Plasmodium falciparum:* Stereo pair of the gametocyte showing subpellicular microtubules (arrows).

that the parasite pellicular structure undergoes major changes during its life cycle and especially that there are major rearrangements in intramembranous particle distribution. Since intramembranous particles are generally regarded as membrane proteins, it follows that significant rearrangements of these proteins occur as the parasite goes from one stage of its life cycle to another. This suggestion is supported by observations that antigens of the different *Plasmodium falciparum* life cycle stages also differ and are stage specific.

REFERENCES

AIKAWA, M., COCHRANE, A. H., NUSSENZWEIG, R. S., AND RABBEGE, J. 1979. Freeze-fracture study of malaria sporozoites: Antibody-induced changes of the pellicular membrane. *Journal of Protozoology* **26**, 273–279.

AIKAWA, M., AND JORDAN, H. B. 1968. Fine structure of a reptilian malarial parasite. *Journal of Parasitology* **54**, 1023–1033.

BRANTON, D., BULLIVANT, S., GILULA, N. B., KARNOVSKY, M. J., MOOR, H., MUHLETHALER, K., NORTHCOTE, D. H., PACKER, L., SATIR, B., SATIR, P., SPETH, V., STAEHLIN, L. A., STEERE, R. L., AND WEINSTEIN, R. S. 1975. Freeze-etching nomenclature. *Science* **190**, 54–56.

DUBREMETZ, J. F., TORPIER, G., MAUROIS, P., PRENSIER, G., AND SINDEN, R. 1979. Structure de la pellicule du sporozoite de *Plasmodium yoelii:* Etude par cryofracture. *Compte Rendu de l'Academie de Science Paris (Series D)* **288**, 623–626.

DUNCAN, D., SHEET, J., JULIAN, S., AND MICHS, D. 1959. Electron microscopic observations of gametocytes of a malarial parasite (*Plasmodium cathemerium*). *Texas Reports on Biology and Medicine* **17**, 314.

GARNHAM, P. C. C., BIRD, R. G., AND BAKER, J. R. 1967. Electron microscope studies of motile stages of malaria parasites. V. Exflagellation in *Plasmodium Hepatocystis* and *Leucocytozoon*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **61**, 58–68.

IFEDIBA, T., AND VANDERBERG, J. P. 1981. Complete *in vitro* maturation of *Plasmodium falciparum* gametocytes. *Nature (London)* **294**, 364–366.

LADDA, R. L., AND STEERE, R. L. 1969. Freeze etching of malarial parasites. *In* "27th Annual Proceedings of the Electron Microscopy Society of America" (C. J. Arcenaux, Ed.), pp. 396–397. Claitor's Publishing Division, Baton Rouge, LA.

MCLAREN, D. J., BANNISTER, L. H., TRIGG, P. I., AND BUTCHER, G. A. 1977. A freeze-fracture study on the parasite-erythrocyte interrelationship in *Plasmodium knowlesi* infections. *Bulletin of the World Health Organization* **55**, 199–203.

MCLAREN, D. J., BANNISTER, L. H., TRIGG, P. I., AND BUTCHER, G. A. 1979. Freeze-fracture studies on the interaction between the malaria parasite and the host erythrocyte in *Plasmodium knowlesi* infections. *Parasitology* **79**, 125–139.

MESZOELY, C. A. M., ERBE, E. F., STEERE, R. L., PACHECO, N. D., AND BEAUDOIN, R. L. 1982. *Plasmodium berghei:* Architectural analysis by freeze-fracturing of the intraoocyst sporozoite's pellicular system. *Experimental Parasitology* **53**, 229–241.

MESZOELY, C. A. M., ERBE, E. F., STEERE, R. L., PALMER, T., AND BEAUDOIN, R. L. 1983. Freeze-etch studies on the gametocytes of *Plasmodium falciparum*. *In* "Forty-First Annual Proceedings of the Electron Microscopy Society of America" (G. W. Gailey, Ed.), pp. 314–315. Claitor's Publishing Division, Baton Rouge, LA.

MESZOELY, C. A. M., STEERE, R. L., AND BAHR, G. F. 1972. Morphologic studies on the freeze-etched avian malarial parasites *Plasmodium gallinaceum*. *In* "Basic Research in Malaria" (E. H. Sadun and A. P. Moon, Eds.). *Proceedings of the Helminthological Society of Washington (Special Issue)* **39**, 149–162.

MESZOELY, C. A. M., STEERE, R. L., ERBE, E. F., AND STEERE, R. L. 1975. Studies on the pellicular complex of the exoerythrocytic merozoites of the avian malarial parasite (*Plasmodium lophurae*). *In* "Thirty-Third Annual Proceedings of the Electron Microscopy Society of America" (G. W. Bailey,

Ed.), pp. 654–661. Claitor's Publishing Division, Baton Rouge, LA.

RUDZINSKA, M. A., AND TRAGER, W. 1968. The fine structure of trophozoites and gametocytes in *Plasmodium vinckei*. *Journal of Protozoology* **15**, 73–88.

SEED, T., PEISTER, R., KREIER, J., AND JOHNSON, A. 1971. *Plasmodium gallinaceum*: Fine structure by freeze-etch technique. *Experimental Parasitology* **30**, 73–81.

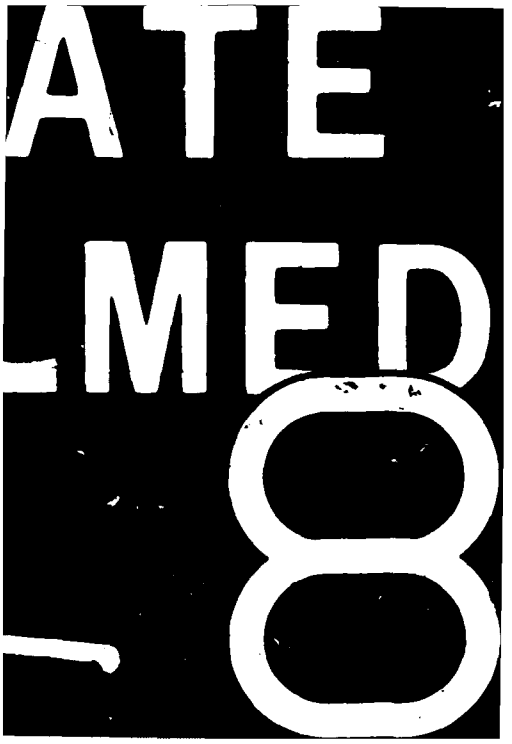
SINDEN, R. E. 1975. Microgametogenesis in *Plasmodium yoelii nigeriensis*: A scanning electron microscope investigation. *Protistologica* **11**, 263–268.

SINDEN, R. E., CANNING, E. U., AND SPAIN, B. 1976. Gametogenesis and fertilization in *Plasmodium yoelii nigeriensis*: A transmission electron microscope study. *Proceedings of the Royal Society of London* **193**, 55–76.

SINDEN, R. E., PONNUDURAI, T., SMITS, M. A., SIMM, A. M., AND MEUWISSEN, J. H. E. TH. 1984. Gametocytogenesis of *Plasmodium falciparum in vitro*: A simple technique for the routine culture of pure capacitated gametocytes *en masse*. *Parasitology* **88**, 239–247.

STEERE, R. L. 1973. Preparation of high resolution freeze-etch, freeze-fracture frozen surface and freeze-dried replicas in a single freeze-etch module and the use of stereo electron microscopy to obtain maximum informaion from them. *In* "Freeze-etching Techniques and Applications" (E. L. Benedetti and P. Favard, Eds.), pp. 223–225. Société française de Microscopie Electronique, Paris.

STERLING, C. R., AND AIKAWA, M. 1973. A comparative study of gametocyte ultrastructure in avian Haemosporida. *Journal of Protozoology* **20**, 81–92.


ATE
MED
8